A

05/14/98 1c559 U.S. PTO

DIVISION-CONTINUATION APPLICATION TRANSMITTAL FORM			Attorney Docket No.: A-451D	
	Anticipated Classification Of This Application: Class	Subclass	Prior Application: Examiner	Art Unit

To the Assistant Commissioner for Patents:

This is a request for filing a ☐ continuation ☒ divisional application, under 37 CFR 1.53(b), of pending prior application Serial No. 08/842,842 filed on April 16 19 97 ,
of William J. Boyle
for Osteoprotegerin Binding Proteins

1. ☒ Enclosed is a copy of the prior application, including the oath or declaration as originally filed. I hereby verify that the attached papers are a true copy of prior application Serial No. **08/842,842** as originally filed on **April 16, 1997**, and further that this Statement was made with the knowledge that willful false statementsand the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United StatesCode and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

2. ☒ The filing fee is calculated below:

For	Number Filed		Number Extra		Rate		Fee
Total Claims	33	- 20 =	13	x	$22.00	=	$ 286.00
Independent Claims	9	- 3 =	6	x	$82.00	=	492.00
Multiple Dependent Claims	0			+	$270.00	=	0.00
Basic Fee					$790.00	=	790.00
					Total Filing Fee		$1,568.00

3. ☒ The Commissioner is hereby authorized to charge any filing fees which may be required by the accompanying application, any additional fees which may be required during pendency of this application, or credit any over-payment to Deposit Account No. 01-0519 in the name of Amgen Inc. An original and one copy are enclosed.

4. ☐ A check in the amount of $ _____ is enclosed.

5. ☐ Cancel in this application original claims _____ of the prior application before calculating the filing fee. (At least one original independent claim must be retained for filing purposes.)

6. ☒ Amend the specification by inserting before the first line the sentence: This application is a

☐ continuation, ☒ division, of application Serial No. **08/842,842**, filed **April 16, 1997** which is hereby incorporated by reference.

EXPRESS MAIL CERTIFICATE

"Express Mail" mail labeling number: TB813684663 Date of Deposit: 5/14/98

I hereby certify that this paper or fee is being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 C F R 1 10 on the date indicated above and is addressed to Box Patent Application, Assistant Commissioner for Patents, Washington, D C 20231

Ellen J. Sorensen (Printed Name) Ellen J. Sorensen (Signature)

7. ☐ Transfer the drawings from the prior application to this application and abandon said prior application as of the filing date accorded this application. A duplicate copy of this sheet is enclosed for filing in the prior application file. (May only be used if signed by person authorized by § 1.138 and before payment of base issue fee.)

7a. ☐ New formal drawings are enclosed.

8. ☐ Priority of application Serial No. _____ filed on _____ in _____ (country) is claimed under 35 U.S.C. 119.

8a. ☐ The certified copy has been filed in prior application Serial No. _____ filed _____

9. ☒ The prior application is assigned of record to **AMGEN INC.**

10. ☐ A preliminary amendment is enclosed.

11. ☒ Also enclosed **PTO Form 1449 and Information Disclosure Statement, Sequence Listing Transmittal and Paper Copy of Sequence Listing**

12. ☒ The power of attorney in the prior application is to:
Ron K. Levy, Registration No.: 31,539; Steven M. Odre, Registration No.: 29,094; and
Robert B, Winter, Registration No.: 34,458

a. ☒ The power appears in the original papers in the prior application.

b. ☐ Since the power does not appear in the original papers, a copy of the power in the prior application is enclosed.

c. ☐ Address all future communications to _____ at the address below.

Signator: ☐ Assignee of complete interest

☒ Attorney or agent of record

Robert B. Winter
Attorney/Agent's Name
Attorney/Agent for Applicant(s)
Registration No. 34,458
Phone: (805) 447-2425
Date: May 14, 1998

Please send all future correspondence to:

U. S. Patent Operations/RBW
Dept. 430, M/S 27-4-A
AMGEN, INC.
One Amgen Center Drive
Thousand Oaks, California 91320-1789

OSTEOPROTEGERIN BINDING PROTEINS

Field of the Invention

The present invention relates to polypeptides which are involved in osteoclast differentiation. More particularly, the invention relates to osteoprotegerin binding proteins, nucleic acids encoding the proteins, expression vectors and host cells for production of the proteins, and binding assays. Compositions and methods for the treatment of bone diseases, such as osteoporosis, bone loss from arthritis, Paget's disease, and hypercalcemia, are also described.

Background of the Invention

Living bone tissue exhibits a dynamic equilibrium between deposition and resorption of bone. These processes are mediated primarily by two cell types: osteoblasts, which secrete molecules that comprise the organic matrix of bone; and osteoclasts, which promote dissolution of the bone matrix and solubilization of bone salts. In young individuals with growing bone, the rate of bone deposition exceeds the rate of bone resorption, while in older individuals the rate of resorption can exceed deposition. In the latter situation, the increased breakdown of bone leads to reduced bone mass and strength, increased risk of fractures, and slow or incomplete repair of broken bones.

Osteoclasts are large phagocytic mutinucleated cells which are formed from hematopoietic precursor cells in the bone marrow. Although the growth and formation of mature functional osteoclasts is not well

understood, it is thought that osteoclasts mature along the monocyte/macrophage cell lineage in response to exposure to various growth-promoting factors. Early development of bone marrow precursor cells to preosteoclasts are believed to mediated by soluble factors such as tumor necrosis factor-α (TNF-α), tumor necrosis factor-β (TNF-β), interleukin-1 (IL-1), interleukin-4 (IL-4), interleukin-6 (IL-6), and leukemia inhibitory factor (LIF). In culture, preosteoclasts are formed in the presence of added macrophage colony stimualting factor (M-CSF). These factors act primarily in early steps of osteoclast development. The involvement of polypeptide factors in terminal stages of osteoclast formation has not been extensively reported. It has been reported, however, that parathyroid hormone stimulates the formation and activity of osteoclasts and that calcitonin has the opposite effect, although to a lesser extent.

Recently, a new polypeptide factor, termed osteoprotegerin (OPG), has been described which negatively regulated formation of osteoclasts in vitro and in vivo (see co-owned and co-pending U.S. Serial Nos. 08/577,788 filed December 22, 1995, 08/706,945 filed September 3, 1996, and 08/771,777, filed December 20, 1996, hereby incorporated by reference; and PCT Application No. WO96/26271). OPG dramatically increased the bone density in transgenic mice expressing the OPG polypeptide and reduced the extent of bone loss when administered to ovariectomized rats. An analysis of OPG activity in in vitro osteoclast formation revealed that OPG does not interfere with the growth and differentiation of monocyte/macrophage precursors, but more likely blocks the differentiation of ostoeclasts from monocyte/macrophage precursors. Thus OPG appears

to have specificity in regulating the extent of osteoclast formation.

OPG comprises two polypeptide domains having different structural and functional properties. The amino-terminal domain spanning about residues 22-194 of the full-length polypeptide (the N-terminal methionine is designated residue 1) shows homology to other members of the tumor necrosis factor receptor (TNFR) family, especially TNFR-2, through conservation of cysteine rich domains characteristic of TNFR family members. The carboxy terminal domain spanning residues 194-401 has no significant homology to any known sequences. Unlike a number of other TNFR family members, OPG appears to be exclusively a secreted protein and does not appear to be synthesized as a membrane associated form.

Based upon its activity as a negative regulator of osteoclast formation, it is postulated that OPG may bind to a polypeptide factor involved in osteoclast differentiation and thereby block one or more terminal steps leading to formation of a mature osteoclast.

It is therefore an object of the invention to identify polypeptides which interact with OPG. Said polypeptides may play a role in osteoclast maturation and may be useful in the treatment of bone diseases.

Summary of the Invention

A novel member of the tumor necrosis factor family has been identified from a murine cDNA library expressed in COS cells screened using a recombinant OPG-Fc fusion protein as an affinity probe. The new polypeptide is a transmembrane OPG binding protein which is predicted to be 316 amino acids in length, and has an amino terminal cytoplasmic domain, a transmembrane doman, and a carboxy terminal extracellular domain. OPG

binding proteins of the invention may be membrane-associated or may be in soluble form.

The invention provides for nucleic acids encoding an OPG binding protein, vectors and host cells expressing the polypeptide, and method for producing recombinant OPG binding protein. Antibodies or fragments thereof which specifically bind OPG binding protein are also provided.

OPG binding proteins may be used in assays to quantitate OPG levels in biological samples, identify cells and tissues that display OPG binding protein, and identify new OPG and OPG binding protein family members. Methods of identifying compounds which interact with OPG binding protein are also provided. Such compounds include nucleic acids, peptides, proteins, carbohydrates, lipids or small molecular weight organic molecules and may act either as agonists or antagonists of OPG binding protein activity.

OPG binding proteins are involved in osteoclast differentiation and the level of osteoclast activity in turn modulates bone resorption. OPG binding protein agonists and antagonists modulate osteoclast formation and bone resorption and may be used to treat bone diseases characterized by changes in bone resorption, such as osteoporosis, hypercalcemia, bone loss due to arthritis or metastasis, Paget's disease, osteopetrosis and the like. Pharmaceutical compositions comprising OPG binding proteins and OPG binding protein agonists and antagonists are also encompassed by the invention.

Description of the Figures

Figure 1. Structure and sequence of the 32D-F3 insert encoding OPG binding protein. Predicted transmembrane domain and sites for asparagine-linked carbohydrate chains are underlined.

Figure 2. OPG binding protein expression in COS-7 cells transfected with pcDNA/32D-F3. Cells were lipofected with pcDNA/32D-F3 DNA, the assayed for binding to either goat anti-human IgG1 alkaline phosphatase conjugate (secondary alone), human OPG[22-201]-Fc plus secondary (OPG-Fc), or a chimeric ATAR extracellular domain-Fc fusion protein (sATAR-Fc). ATAR is a new member of the TNFR superfamily, and the sATAR-Fc fusion protein serves as a control for both human IgG1 Fc domain binding, and generic TNFR releated protein, binding to 32D cell surface molecules.

Figure 3. Expression of OPG binding protein in human tissues. Northern blot analysis of human tissue mRNA (Clontech) using a radiolabeled 32D-F3 derived hybridization probe. Relative molecular mass is indicated at the left in kilobase pairs (kb). Arrowhead on right side indicates the migration of an approximately 2.5 kb transcript detected in lymph node mRNA. A very faint band of the same mass is also detected in fetal liver.

Detailed Description of the Invention

The invention provides for a polypeptide referred to as an OPG binding protein, which specficially binds OPG and is involved in osteoclast differentiation. A cDNA clone encoding the murine form

of the polypeptide was identified from a library prepared from a mouse myelomonocytic cell line 32-D and transfected into COS cells. Transfectants were screened for their ability to bind to an OPG[22-201]-Fc fusion polypeptide (Example 1). The nucleic acid sequence revealed that OPG binding protein is a novel member of the TNF receptor family and is most closely related to AGP-1, a polypeptide previously described in co-owned and co-pending U.S. Serial No. 08/660,562, filed June 7, 1996. (A polypeptide identical to AGP-1 and designated TRAIL is described in Wiley et al. Immunity 3, 673-682 (1995)). OPG binding protein is predicted to be a type II transmembrane protein having a cytoplamsic domain at the amino terminus, a transmembrane domain, and a carboxy terminal extracellular domain (Figure 1). The amino terminal cytoplasmic domain spans about residues 1-48, the transmembrane domain spans about residues 49-69 and the extracellular domain spans about residues 70-316 as shown in Figure 1 (SEQ ID NO:___). The membrane-associated protein specifically binds OPG (Figure 2). Thus OPG binding protein and OPG share many characteristics of a receptor-ligand pair although it is possible that other naturally-occurring ligands for OPG binding protein exist.

OPG binding protein refers to a polypeptide having an amino acid sequence of mammalian OPG binding protein, or a fragment, analog, or derivative thereof, and having at least the activity of binding OPG. In preferred embodiments, OPG binding protein is of murine or human origin. In another embodiment, OPG binding protein is a soluble protein having, in one form, an isolated extracellular domain separate from cytoplasmic and transmembrane domains. OPG binding protein is involved in osteoclast differentiation and in the rate and extent of bone resorption.

Nucleic Acids

The invention provides for isolated nucleic acids encoding OPG binding proteins. As used herein, the term nucleic acid comprises cDNA, genomic DNA, wholly or partially synthetic DNA or RNA. The nucleic acids of the invention are selected from the group consisting of:

a) the nucleic acids as shown in Figure 1 (SEQ ID NO: ___);

b) nucleic acids which hybridize to the polypeptide coding regions of the nucleic acids shown in Figure 1 (SEQ ID NO:___) and remain hybridized to the nucleic acids under high stringency conditions; and

c) nucleic acids which are degenerate to the nucleic acids of (a) or (b).

Nucleic acid hybridizations typically involve a multi-step process comprising a first hybridization step to form nucleic acid duplexes from single strands followed by a second hybridization step carried out under more stringent conditions to selectively retain nucleic acid duplexes having the desired homology. The conditions of the first hybridization step are generally not crucial, provided they are not of higher stringency than the second hybridization step. Generally, the second hybridization is carried out under conditions of high stringency, wherein "high stringency" conditions refers to conditions of temperature and salt which are about 12-20°C below the melting temperature (T_m) of a perfect hybrid of part or all of the complementary strands corresponding to Figure 1 (SEQ. ID. NO: ___). In one embodiment, "high stringency" conditions refer to conditions of about 65°C and not more than about 1M Na+. It is understood that salt concentration, temperature and/or length of incubation may be varied in either the

first or second hybridization steps such that one obtains the hybridizing nucleic acid molecules according to the invention. Conditions for hybridization of nucleic acids and calculations of T_m for nucleic acid hybrids are described in Sambrook et al. Molecular Cloning: A Laboratory Manual Cold Spring Harbor Laboratory Press, New York (1989).

The nucleic acids of the invention may hybridize to part or all of the polypeptide coding regions of OPG binding protein as shown in Figure 1 (SEQ ID NO: ___) and therefore may be truncations or extensions of the nucleic shown therein. Truncated or extended nucleic acids are encompassed by the invention provided that they retain at least the property of binding OPG. In one embodiment, the nucleic acid will encode a polypeptide of at least about 10 amino acids. In another embodiment, the nucleic acid will encode a polypeptide of at least about 20 amino acids. In yet another embodiment, the nucleic acid will encode a polypeptide of at least about 50 amino acids. The hybridizing nucleic acids may also include noncoding sequences located 5' and/or 3' to the OPG binding protein coding regions. Noncoding sequences include regulatory regions involved in expression of OPG binding protein, such as promoters, enhancer regions, translational initiation sites, transcription termination sites and the like.

In preferred embodiments, the nucleic acids of the invention encode mouse or human OPG binding protein. Nucleic acids may encode a membrane bound form of OPG binding protein or soluble forms which lack a functional transmembrane region. The predicted transmembrane region for murine OPG binding protein includes amino acid residues 49-69 inclusive as shown in Figure 1 (SEQ. ID. NO: ___). Substitutions which replace hydrophobic amino acid residues in this region with neutral or

hydrophilic amino acid residues would be expected to disrupt membrane association and result in soluble OPG binding protein. In addition, deletions of part or all the transmembrane region would also be expected to produce soluble forms of OPG binding protein. Nucleic acids encoding amino acid residues 70-316 as shown in Figure 1 (SEQ ID NO:___), or fragments and analogs thereof, encompass soluble OPG binding protein.

Nucleic acid sequences of the invention may be used for the detection of sequences encoding OPG binding protein in biological samples. In particular, the sequences may be used to screen cDNA and genomic libraries for related OPG binding protein sequences, especially those from other species. The nucleic acids are also useful for modulating levels of OPG binding protein by anti-sense technology or in vivo gene expression. Development of transgenic animals expressing OPG binding protein is useful for production of the polypeptide and for the study of in vivo biological activity.

Vectors and Host Cells

The nucleic acids of the invention will be linked with DNA sequences so as to express biologically active OPG binding protein. Sequences required for expression are known to those skilled in the art and include promoters and enhancer sequences for initiation of RNA synthesis, transcription termination sites, ribosome binding sites for the initiation of protein synthesis, and leader sequences for secretion. Sequences directing expression and secretion of OPG binding protein may be homologous, i.e., the sequences are identical or similar to those sequences in the genome involved in OPG binding protein expression and secretion, or they may be heterologous. A variety of plasmid vectors are available for expressing OPG binding

protein in host cells (see, for example, Methods in Enzymology v. 185, Goeddel, D.V. ed., Academic Press (1990)). For expression in mammalian host cells, a preferred embodiment is plasmid pDSRα described in PCT Application No. 90/14363. For expression in bacterial host cells, preferred embodiments include plasmids harboring the lux promoter (see co-owned and co-pending U.S. Serial No. 08/577,778, filed December 22, 1995). In addition, vectors are available for the tissue-specific expression of OPG binding protein in transgenic animals. Retroviral and adenovirus-based gene transfer vectors may also be used for the expression of OPG binding protein in human cells for in vivo therapy (see PCT Application No. 86/00922).

Procaryotic and eucaryotic host cells expressing OPG binding protein are also provided by the invention. Host cells include bacterial, yeast, plant, insect or mammalian cells. OPG binding protein may also be produced in transgenic animals such as mice or goats. Plasmids and vectors containing the nucleic acids of the invention are introduced into appropriate host cells using transfection or transformation techniques known to one skilled in the art. Host cells may contain DNA sequences encoding OPG binding protein as shown in Figure 1 or a portion thereof, such as the extracellular domain or the cytoplasmic domain. Nucleic acids encoding OPG binding proteins may be modified by substitution of codons which allow for optimal expression in a given host. At least some of the codons may be so-called preference codons which do not alter the amino acid sequence and are frequently found in genes that are highly expressed. However, it is understood that codon alterations to optimize expression are not restricted to the introduction of preference codons. Examples of preferred mammalian host cells for OPG binding protein expression include, but are not

limited to COS, CHOd-, 293 and 3T3 cells. A preferred bacterial host cell is Escherichia coli.

Polypeptides

The invention also provides OPG binding protein as the product of procaryotic or eucaryotic expression of an exogenous DNA sequence, i.e., OPG binding protein is recombinant OPG binding protein. Exogenous DNA sequences include cDNA, genomic DNA and synthetic DNA sequences. OPG binding protein may be the product of bacterial, yeast, plant, insect or mammalian cells expression, or from cell-free translation systems. OPG binding protein produced in bacterial cells will have an N-terminal methionine residue. The invention also provides for a process of producing OPG binding protein comprising growing procaryotic or eucaryotic host cells transformed or transfected with nucleic acids encoding OPG binding protein and isolating polypeptide expression products of the nucleic acids.

Polypeptides which are mamalian OPG binding protein or are fragments, analogs or derivatives thereof are encompassed by the invention. A fragment of OPG binding protein refers to a polypeptide having a deletion of one or more amino acids such that the resulting polypeptide has at least the property of binding OPG. Said fragments will have deletions originating from the amino terminal end, the carboxy terminal end, and internal regions of the polypeptide. Fragments of OPG binding protein are at least about ten amino acids, at least about 20 amino acids, or at least about 50 amino acids in length. In preferred embodiments, OPG binding protein will have a deletion of one or more amino acids from the transmembrane region (amino acid residues 49-69 as shown in Figure 1), or, alternatively, one or more amino acids from the amino-terminus up to and/or including the transmembrane

region (amino acid residues 1-49 as shown in Figure 1). In another embodiment, OPG binding protein is a soluble protein comprising, for example, amino acid residues 70-316, or N-terminal or C-terminal truncated forms thereof, which retain OPG binding activity. An analog of an OPG binding protein refers to a polypeptide having a substitution or addition of one or more amino acids such that the resulting polypeptide has at least the property of binding OPG. Said analogs will have substitutions or additions at any place along the polypeptide. Preferred analogs include those of soluble OPG binding proteins. Fragments or analogs may be naturally occurring, such as a polypeptide product of an allelic variant or a mRNA splice variant, or they may be constructed using techniques available to one skilled in the art for manipulating and synthesizing nucleic acids. The polypeptides may or may not have an amino terminal methionine residue

Also included in the invention are derivatives of OPG binding protein which are polypeptides that have undergone post-translational modifications (e.g., addition of N-linked or O-linked carbohydrate chains, processing of N-terminal or C-terminal ends), attachment of chemical moieties to the amino acid backbone, chemical modifications of N-linked or O-linked carbohydrate chains, and addition of an N-terminal methionine residue as a result of procaryotic host cell expression. In particular, chemically modified derivatives of OPG binding protein which provide additional advantages such as increased stability, longer circulating time, or decreased immunogenicity are contemplated. Of particular use is modification with water soluble polymers, such as polyethylene glycol and derivatives thereof (see for example U.S. Patent No. 4,179,337). The chemical moieties for derivitization may be selected from water soluble

polymers such as polyethylene glycol, ethylene glycol/propylene glycol copolymers, carboxymethylcellulose, dextran, polyvinyl alcohol and the like. The polypeptides may be modified at random positions within the molecule, or at predetermined positions within the molecule and may include one, two, three or more attached chemical moieties. Polypeptides may also be modified at pre-determined positions in the polypeptide, such as at the amino terminus, or at a selected lysine or arginine residue within the polypeptide. Other chemical modificaitons provided include a detectable label, such as an enzymatic, fluorescent, isotopic or affinity label to allow for detection and isolation of the protein.

OPG binding protein chimeras comprising part or all of an OPG binding protein amino acid sequence fused to a heterologous amino acid sequence are also included. The heterologous sequence may be any sequence which allows the resulting fusion protein to retain the at least the activity of binding OPG. In a preferred embodiment, the carboxy terminal extracellular domain of OPG binding protein is fused to a heterologous sequence. Such sequences include heterologous cytoplasmic domains that allow for alternative intracellular signalling events, sequences which promote oligomerization such as the Fc region of IgG, enzyme sequences which provide a label for the polypeptide, and sequences which provide affinity probes, such as an antigen-antibody recognition.

The polypeptides of the invention are isolated and purified from tissues and cell lines which express OPG binding protein, either extracted from lysates or from conditioned growth medium, and from transformed host cells expressing OPG binding protein. OPG binding protein may be obtained from murine myelomonocytic cell line 32-D (ATCC accession no. CRL-11346). Human OPG

binding protein, or nucleic acids encoding same, may be isolated from human lymph node or fetal liver tissue. Isolated OPG binding protein is free from association with human proteins and other cell constituents.

A method for the purification of OPG binding protein from natural sources (e.g. tissues and cell lines which normally express OPG binding protein) and from transfected host cells is also encompassed by the invention. The purification process may employ one or more standard protein purification steps in an appropriate order to obtain purified protein. The chromatography steps can include ion exchange, gel filtration, hydrophobic interaction, reverse phase, chromatofocusing, affinity chromatography employing an anti-OPG binding protein antibody or biotin-streptavidin affinity complex and the like.

Antibodies

Antibodies specifically binding the polypeptides of the invention are also encompassed by the invention. The antibodies may be produced by immunization with full-length OPG binding protein, soluble forms of OPG binding protein, or a fragment thereof. The antibodies of the invention may be polyclonal or monoclonal, or may be recombinant antibodies, such as chimeric antibodies wherein the murine constant regions on light and heavy chains are replaced by human sequences, or CDR-grafted antibodies wherein only the complementary determining regions are of murine origin. Antibodies of the invention may also be human antibodies prepared, for example, by immunization of transgenic animals capable of producing human antibodies (see, for example, PCT Application No. WO93/12227). The antibodies are useful for detecting OPG binding protein in biological samples, thereby allowing the identification of cells or tissues

which produce the protein In addition, antibodies which bind to OPG binding protein and block interaction with other binding compounds may have therapeutic use in modulating osteoclast differentiation and bone resorption.

Compositions

The invention also provides for pharmaceutical compositions comprising a therapeutically effective amount of the OPG binding protein of the invention together with a pharmaceutically acceptable diluent, carrier, solubilizer, emulsifier, preservative and/or adjuvant. The invention also provides for pharmaceutical compositions comprising a therapeutically effective amount of an OPG binding protein agonist or antagonist. The term "therapeutically effective amount" means an amount which provides a therapeutic effect for a specified condition and route of administration. The composition may be in a liquid or lyophilized form and comprises a diluent (Tris, acetate or phosphate buffers) having various pH values and ionic strengths, solubilizer such as Tween or Polysorbate, carriers such as human serum albumin or gelatin, preservatives such as thimerosal or benzyl alcohol, and antioxidants such as ascrobic acid or sodium metabisulfite. Selection of a particular composition will depend upon a number of factors, including the condition being treated, the route of administration and the pharmacokinetic parameters desired. A more extensive survey of component suitable for pharmaceutical compositions is found in Remington's Pharmaceutical Sciences, 18th ed. A.R. Gennaro, ed. Mack, Easton, PA (1980).

In a preferred embodiment, compositions comprising soluble OPG binding proteins are also provided. Also encompassed are compositions comprising soluble OPG binding protein modified with water soluble

polymers to increase solubility, stability, plasma half-life and bioavailability. Compositions may also comprise incorporation of soluble OPG binding protein into liposomes, microemulsions, micelles or vesicles for controlled delivery over an extended period of time. Soluble OPG binding protein may be formulated into microparticles suitable for pulmonary administration.

Compositions of the invention may be administered by injection, either subcutaneous, intravenous or intramuscular, or by oral, nasal, pulmonary or rectal administration. The route of administration eventually chosen will depend upon a number of factors and may be ascertained by one skilled in the art.

The invention also provides for pharmaceutical compositions comprising a therapeutically effective amount of the nucleic acids of the invention together with a pharmaceutically acceptable adjuvant. Nucleic acid compositions will be suitable for the delivery of part or all of the coding region of OPG binding protein and/or flanking regions to cells and tissues as part of an anti-sense therapy regimen.

Methods of Use

OPG binding proteins may be used in a variety of assays for detecting OPG and characterizing interactions with OPG. In general, the assay comprises incubating OPG binding protein with a biological sample containing OPG under conditions which permit binding to OPG to OPG binding protein, and measuring the extent of binding. OPG may be purified or present in mixtures, such as in body fluids or culture medium. Assays may be developed which are qualitative or quantitative, with the latter being useful for determining the binding parameters (affinity constants and kinetics) of OPG to OPG binding protein and for quantitating levels of

biologically active OPG in mixtures. Assays may also be used to evaluate the binding of OPG tc fragments, analogs and derivatives of OPG binding protein and to identify new OPG and OPG binding protein family members.

Binding of OPG to OPG binding protein may be carried out in several formats, including cell-based binding assays, membrane binding assays, solution-phase assays and immunoassays. In general, trace levels of labeled OPG are incubated with OPG binding protein samples for a specified period of time followed by measurement of bound OPG by filtration, electrochemiluminescent (ECL, ORIGEN system by IGEN), cell-based or immunoassays. Homogeneous assay technologies for radioactivity (SPA; Amersham) and time resolved fluoresence (HTRF, Packard) can also be implemented. Binding is detected by labeling OPG or an anti-OPG antibody with radioactive isotopes (125I, 35S, 3H), fluorescent dyes (fluorescein), lanthanide (Eu3+) chelates or cryptates, orbipyridyl-ruthenium (Ru2+) complexes. It is understood that the choice of a labeled probe will depend upon the detection system used. Alternatively, OPG may be modified with an unlabled epitope tag (e.g., biotin, peptides, His_6, myc) and bound to proteins such as streptavidin, anti-peptide or anti-protein antibodies which have a detectable label as described above.

In an alternative method, OPG binding protein may be assayed directly using polyclonal or monoclonal antibodies to OPG binding proteins in an immunoassay. Additional forms of OPG binding proteins containing epitope tags as described above may be used in solution and immunoassays.

Methods for indentifying compounds which interact with OPG binding protein are also encompassed by the invention. The method comprises incubating OPG binding protein with a compound under conditions which

permit binding of the compound to OPG binding protein, and measuring the extent of binding. The compound may be substantially purified or present in a crude mixture. Binding compounds may be nucleic acids, proteins, peptides, carbohydrates, lipids or small molecular weight organic compounds. The compounds may be further characterized by their ability to increase or decrease OPG binding protein activity in order to determine whether they act as an agonist or an antagonist.

OPG binding proteins are also useful for identification of intracellular proteins which interact with the cytoplasmic domain by a yeast two-hybrid screening process. As an example, hybrid constructs comprising DNA encoding the N-terminal 50 amino acids of an OPG binding protein fused to a yeast GAL4-DNA binding domain may be used as a two-hybrid bait plasmid. Positive clones emerging from the screening may be characterized further to identify interacting proteins. This information may help elucidate a intracellular signaling mechanism associated with OPG binding protein and provide intracellular targets for new drugs that modulate bone resorption.

The invention also encompasses modulators (agonists and antagonists) of OPG binding protein and the methods for obtaining them. An OPG binding protein modulator may either increase or decrease at least one activity associated with OPG binding protein, such as ability to bind OPG or some other interacting molecule or to regulate osteoclast maturation. Typically, an agonist or antagonist may be a co-factor, such as a protein, peptide, carbohydrate, lipid or small molecular weight molecule, which interacts with OPG binding protein to regulate its activity. Potential polypeptide antagonists include antibodies which react with either soluble or membrane-associated forms of OPG binding protein, and soluble forms of OPG binding protein which

comprise part or all of the extracellular domain of OPG binding protein. Molecules which regulate OPG binding protein expression typically include nucleic acids which are complementary to nucleic acids encoding OPG binding protein and which act as anti-sense regulators of expression.

OPG binding protein is involved in controlling formation of mature osteoclasts, the primary cell type implicated in bone resorption. An increase in the rate of bone resorption (over that of bone formation) can lead to various bone disorders collectively referred to as osteopenias, and include osteoporosis, osteomyelitis, hypercalcemia, osteopenia brought on by surgery or steroid administration, Paget's disease, osteonecrosis, bone loss due to rheumatoid arthritis, periodontal bone loss, and osteolytic metastasis. Conversely, a decrease in the rate of bone resportion can lead to osteopetrosis, a condition marked by excessive bone density. Agonists and antagonists of OPG binding protein modulate osteoclast formation and may be administered to patients suffering from bone disorders. Agonists and antagonists of OPG binding protein used for the treatment of osteopenias may be administered alone or in combination with a therapeutically effective amount of a bone growth promoting agent including bone morphogenic factors designated BMP-1 to BMP-12, transforming growth factor-β and TGF-β family members, interleukin-1 inhibitors, TNFα inhibitors, parathyroid hormone, E series prostaglandins, bisphosphonates and bone-enhancing minerals such as fluoride and calcium.

The following examples are offered to more fully illustrate the invention, but are not construed as limiting the scope thereof.

Example 1

Identification of a cell line source for an OPG binding protein

Osteoprotegerin (OPG) negatively regulates osteoclastogenesis in vitro and in vivo. Since OPG is a TNFR-related protein, it is likely to interact with a TNF-related family member while mediating its effects. With one exception, all known members of the TNF superfamily are type II transmembrane proteins expressed on the cell surface. To identify a source of an OPG binding protein, recombinant OPG-Fc fusion proteins were used as immunoprobes to screen for OPG binding proteins located on the surface of various cell lines and primary hematopoietic cells.

Cell lines that grew as adherent cultures in vitro were treated using the following methods: Cells were plated into 24 well tissue culture plates (Falcon), then allowed to grow to approxiamtely 80% confluency. The growth media was then removed, and the adherent cultures were washed with phosphate buffered saline (PBS) (Gibco) containing 1% fetal calf serum (FCS). Recombinant mouse OPG [22-194]-Fc and human OPG [22-201]-Fc fusion proteins (see U.S. Serial No. 08/706,945 filed September 3, 1996) were individually diluted to 5 ug/ml in PBS containing 1% FCS, then added to the cultures and allowed to incubate for 45 min at 0°C. The OPG-Fc fusion protein solution was discarded, and the cells were washed in PBS-FCS solution as described above. The cultures were then exposed to phycoeyrthrin-conguated goat F(ab') anti-human IgG secondary antibody (Southern Biotechnology Associates Cat. # 2043-09) diluted into PBS-FCS. After a 30-45 min incubation at 0°C, the solution was discarded, and the cultures were washed as described above. The cells were

then analysed by immunofluorescent microscopy to detect cell lines which express a cell surface OPG binding protein.

Suspension cell cultures were analysed in a similar manner with the following modifications: The diluent and wash buffer consisted of calcium- and magnesium-free phosphate buffered saline containing 1% FCS. Cells were harvested from exponentially replicating cultures in growth media, pelleted by centrifugation, then resuspended at 1 X 107 cells/ml in a 96 well microtiter tissue culture plate (Falcon). Cells were sequentially exposed to recombinant OPG-Fc fusion proteins, then secondary antibody as described above, and the cells were washed by centrifugation between each step. The cells were then analysed by fluorescence activated cell sorting (FACS) using a Becton Dickinson FACscan.

Using this approach, the murine myelomonocytic cell line 32D (ATCC accession no. CRL-11346) was found to express a surface molecule which could be detected with both the mouse OPG[22-194]-Fc and the human OPG[22-201]-Fc fusion proteins. Secondary antibody alone did not bind to the surface of 32D cells nor did purified human IgG1 Fc, indicating that binding of the OPG-Fc fusion proteins was due to the OPG moiety. This binding could be competed in a dose dependent manner by the addition of recombinant murine or human OPG[22-401] protein. Thus the OPG region required for its biological activity is capable of specifically binding to a 32D-derived surface molecule.

Example 2

Expression cloning of a murine OPG binding protein

A cDNA library was prepared from 32D mRNA, and ligated into the mammalian expression vector pcDNA3.1(+) (Invitrogen, San Diego, CA). Exponentially growing 32D cells maintained in the presence of recombinant interleukin-3 were harvested, and total cell RNA was purified by acid guanidinium thiocyanate-phenol-chloroform extraction (Chomczynski and Sacchi. Anal. Biochem. 162, 156-159, (1987)). The poly (A+) mRNA fraction was obtained from the total RNA preparation by adsorption to, and elution from, Dynabeads Oligo (dT)25 (Dynal Corp) using the manufacturer's recommended procedures. A directional, oligo-dT primed cDNA library was prepared using the Superscript Plasmid System (Gibco BRL, Gaithersburg, Md) using the manufacturer's recommended procedures. The resulting cDNA was digested to completion with Sal I and Not I restriction endonuclease, then fractionated by size exclusion gel chromatography. The highest molecular weight fractions were selected, and then ligated into the polyliker region of the plasmid vector pcDNA3.1(+) (Invitrogen, San Diego, CA). This vector contains the CMV promotor upstream of multiple cloning site, and directs high level expression in eukaryotic cells. The library was then electroporated into competent E. coli (ElectroMAX DH10B, Gibco, NY), and titered on LB agar containing 100 ug/ml ampicillin. The library was then arrayed into segregated pools containing approximately 1000 clones/pool, and 1.0 ml cultures of each pool were grown for 16-20 hr at 37°C. Plasmid DNA from each culture was prepared using the Qiagen Qiawell 96 Ultra Plasmid Kit (catalog #16191) following manufacturer's recommended procedures.

Arrayed pools of 32D cDNA expression library were individually lipofected into COS-7 cultures, then assayed for the acquisition of a cell surface OPG binding protein. To do this, COS-7 cells were plated at a density of $1X10^6$ per ml in six-well tissue culture plates (Costar), then cultured overnight in DMEM (Gibco) containing 10% FCS. Approximately 2 µg of plasmid DNA from each pool was diluted into 0.5 ml of serum-free DMEM, then sterilized by centrifugation through a 0.2 µm Spin-X column (Costar). Simultaneously, 10 µl of Lipofectamine (Life Technologies Cat # 18324-012) was added to a separate tube containing 0.5ml of serum-free DMEM. The DNA and Lipofectamine solutions were mixed, and allowed to incubate at RT for 30 min. The COS-7 cell cultures were then washed with serum-free DMEM, and the DNA-lipofectamine complexes were exposed to the cultures for 2-5 hr at 37°C. After this period, the media was removed, and replaced with DMEM containing 10%FCS. The cells were then cultured for 48 hr at 37°C.

To detect cultures that express an OPG binding protein, the growth media was removed, and the cells were washed with PBS-FCS solution. A 1.0 ml volume of PBS-FCS containing 5 µg/ml of human OPG[22-201]-Fc fusion protein was added to each well and incubated at RT for 1 hr. The cells were washed three times with PBS-FCS solution, and then fixed in PBS containing 2% paraformaldehyde and 0.2% glutaraldehyde in PBS at RT for 5 min. The cultures were washed once with PBS-FCS, then incubated for 1 hr at 65°C while immersed in PBS-FCS solution. The cultures were allowed to cool, and the PBS-FCS solution was aspirated. The cultures were then incubated with an alkaline-phosphatase conjugated goat anti-human IgG (Fc specific) antibody (SIGMA Product # A-9544) at Rt for 30 min, then washed

three-times with 20 mM Tris-Cl (pH 7.6), and 137 mM NaCl. Immune complexes that formed during these steps were detected by assaying for alkaline phosphatase activity using the Fast Red TR/AS-MX Substrate Kit (Pierce, Cat. # 34034) following the manufacturer's recommended procedures.

Using this approach, a total of approximately 300,000 independent 32D cDNA clones were screened, represented by 300 transfected pools of 1000 clones each. A single well was identifed that contained cells which acquired the ability to be specifically decorated by the OPG-Fc fusion protein. This pool was subdivided by sequential rounds of sib selection, yeilding a single plasmid clone 32D-F3 (Figure 1). 32D-F3 plasmid DNA was then transfected into COS-7 cells, which were immunostained with either FITC-conjugated goat anti-human IgG secondary antibody alone, human OPG[22-201]-Fc fusion protein plus secondary, or with ATAR-Fc fusion protein (ATAR also known as HVEM; Montgomery et al. Cell 87, 427-436 (1996)) (Figure 2). The secondary antibody alone did not bind to COS-7/32D-F3 cells, nor did the ATAR-Fc fusion protein. Only the OPG Fc fusion protein bound to the COS-7/32D-F3 cells, indicating that 32D-F3 encoded an OPG binding protein displayed on the surface of expressing cells.

Example 3

OPG Binding Protein Sequence

The 32D-F3 clone isolated above contained an approximately 2.3 kb cDNA insert (Figure 1), which was sequenced in both directions on an Applied Biosystems 373A automated DNA sequencer using primer-driven Taq dye-terminator reactions (Applied Biosystems) following the manufacturer's recommended procedures. The

resulting nucleotide sequence obtained was compared to the DNA sequence database using the FASTA program (GCG, Univeristy of Wisconsin), and analysed for the presence of long open reading frames (LORF's) using the "Six-way open reading frame" application (Frames) (GCG, Univeristy of Wisconsin). A LORF of 316 amino acid (aa) residues beginning at methionine was detected in the appropriate orientation, and was preceded by a 5' untranslated region of about 150 bp. The 5' untranslated region contained an in-frame stop codon upstream of the predicted start codon. This indicates that the structure of the 32D-F3 plasmid is consistent with its ability to utilize the CMV promotor region to direct expression of a 316 aa gene product in mammalian cells.

The predicted OPG binding protein sequence was then compared to the existing database of known protein sequences using a modified version of the FASTA program (Pearson, Meth. Enzymol. 183, 63-98 (1990)). The amino acid sequence was also analysed for the presence of specific motifs conserved in all known members of the tumor necrosis factor (TNF) superfamily using the sequence profile method of (Gribskov et al. Proc. Natl. Acad. Sci. USA 83, 4355-4359 (1987)), as modified by Lüethy et al. Protein Sci. 3, 139-146 (1994)). There appeared to be significant homology throughout the OPG binding protein to several members of the TNF superfamily. The mouse OPG binding protein appear to be most closely related to the mouse and human homologs of both TRAIL and CD40. Further analysis of the OPG binding protein sequence indicated a strong match to the TNF superfamily, with a highly significant Z score of 19.46.

The OPG binding protein aa sequence contains a probable hydrophobic transmembrane domain that begins at

a M49 and extends to L69. Based on this configuration relative to the methionine start codon, the OPG binding protein is predicted to be a type II transmembrane protein, with a short N-terminal intracellular domain, and a longer C-terminal extracellular domain (Figure 4). This would be similar to all known TNF family members, with the exception of lymphotoxin alpha (Nagata and Golstein, Science 267, 1449-1456 (1995)).

Example 4

Expression of human OPG binding protein mRNA

Multiple human tissue northern blots (Clontech, Palo Alto, CA) were probed with a ^{32}P-dCTP labelled 32D-F3 restriction fragment to detect the size of the human transcript and to determine patterns of expression. Northern blots were prehybridized in 5X SSPE, 50% formamide, 5X Denhardt's solution, 0.5% SDS, and 100 µg/ml denatured salmon sperm DNA for 2-4 hr at 42°C. The blots were then hybridized in 5X SSPE, 50% formamide, 2X Denhardt's solution, 0.1% SDS, 100 µg/ml denatured salmon sperm DNA, and 5 ng/ml labelled probe for 18-24 hr at 42°C. The blots were then washed in 2X SSC for 10 min at RT, 1X SSC for 10 min at 50°C, then in 0.5X SSC for 10-15 min.

Using a probe derived from the mouse cDNA and hybridization under stringent conditions, a predominant mRNA species with a relative molecular mass of about 2.5 kb was detected in lymph nodes (Figure 3). A faint signal was also detected at the same relative molecular mass in fetal liver mRNA. No OPG binding protein transcripts were detected in the other tissues examined. The data suggest that expression of OPG binding protein mRNA was extremely restricted in human tissues. The

data also indicate that the cDNA clone isolated is very close to the size of the native transcript, suggesting 32D-F3 is a full length clone.

Example 5

Molecular cloning of the human OPG binding protein

The human homolog of the OPG binding protein is expressed as an approximately 2.5 kb mRNA in human peripheral lymph nodes and is detected by hybridization with a mouse cDNA probe under stringent hybdization conditions. DNA encoding human OPG binding protein is obtained by screening a human lymph node cDNA library by either recombinant bacteriphage plaque, or transformed bacterial colony, hybridiziation methods (Sambrook et al. Molecular Cloning: A Laboratory Manual Cold Spring Harbor Press, New York (1989)). To this the phage or plasmid cDNA library are screened using radioactively-labeled probes derived from the murine OPG binding protein clone 32D-F3. The probes are used to screen nitrocellulose filter lifted from a plated library. These filters are prehybridized and then hybridized using conditions specified in Example 4, ultimately giving rise to purified clones of the human OPG binding protein cDNA. Inserts obtained from any human OPG binding protein clones would be sequenced and analysed as described in Example 3.

Example 6

Cloning and Bacterial Expression of OPG binding protein

PCR amplification employing the primer pairs and templates described below are used to generate various forms of human OPG binding proteins. One primer of each pair introduces a TAA stop codon and a unique SacII site following the carboxy terminus of the gene. The other primer of each pair introduces a unique NdeI site, a N-terminal methionine, and optimized codons for the amino terminal portion of the gene. PCR and thermocycling is performed using standard recombinant DNA methodology. The PCR products are purified, restriction digested, and inserted into the unique NdeI and SacII sites of vector pAMG21 (ATCC accession no. 98113) and transformed into the prototrophic E. coli 393. Other commonly used E. coli expression vectors and host cells are also suitable for expression. After transformation, the clones are selected, plasmid DNA is isolated and the sequence of the OPG binding protein insert is confirmed.

pAMG21-Murine OPG binding protein [75-316]

This construct is engineered to be 242 amino acids in length and have the following N-terminal and C-terminal residues, NH_2-Met(75)-Asp-Pro-Asn-Arg-------Gln-Asp-Ile-Asp(316)-COOH. The template to be used for PCR is pcDNA/32D-F3 and oligonucleotides #1581-72 and #1581-76 will be the primer pair to be used for PCR and cloning this gene construct.

1581-72:

5'-GTTCTCCTCATATGGATCCAAACCGTATTTCTGAAGACAGCACTCACTGCTT-3'

(SEQ ID NO:___)

1581-76:

5'-TACGCACTCCGCGGTTAGTCTATGTCCTGAACTTTGA-3'

(SEQ ID NO:___)

pAMG21-Murine OPG binding protein [158-316]

This construct is engineered to be 160 amino acids in length and have the following N-terminal and C-terminal residues, NH_2-Met-Lys(158)-Pro-Glu-Ala-------Gln-Asp-Ile-Asp(316)- COOH. The template to be used for PCR is pcDNA/32D-F3 and oligonucleotides #1581-73 and #1581-76 will be the primer pair to be used for PCR and cloning this gene construct.

1581-73:

5'-GTTCTCCTCATATGAAACCTGAAGCTCAACCATTTGCACACCTCACCATCAAT-3'

(SEQ ID NO:___)

1581-76:

5'-TACGCACTCCGCGGTTAGTCTATGTCCTGAACTTTGA-3'

(SEQ ID NO:___)

pAMG21-Murine OPG binding protein [166-316]

This construct is engineered to be 152 amino acids in length and have the following N-terminal and C-terminal residues, NH_2-Met-His(166)-Leu-Thr-Ile-------Gln-Asp-Ile-Asp(316)- COOH. The template to be used for PCR is pcDNA/32D-F3 and oligonucleotides #1581-75 and #1581-76 will be the primer pair to be used for PCR and cloning this gene construct.

1581-75:

5'-GTTCTCCTCATATGCATTTAACTATTAACGCTGCATCTATCCCAT CGGGTTCCCATAAAGTCACT-3' (SEQ ID NO:___)

1581-76:

5'-TACGCACTCCGCGGTTAGTCTATGTCCTGAACTTTGA-3' (SEQ ID NO:___)

pAMG21-Murine OPG binding protein [168-316]

This construct is engineered to be 150 amino acids in length and have the following N-terminal and C-terminal residues, NH_2-Met-Thr(168)-Ile-Asn-Ala-------Gln-Asp-Ile-Asp(316)- COOH. The template to be used for PCR is pcDNA/32D-F3 and oligonucleotides #1581-74 and #1581-76 will be the primer pair to be used for PCR and cloning.

1581-74:
5'-GTTCTCCTCATATGACTATTAACGCTGCATCTATCCCATCGGGTTCCCATAAAGTCACT-3' (SEQ ID NO:___)

1581-76:
5'-TACGCACTCCGCGGTTAGTCTATGTCCTGAACTTTGA-3' (SEQ ID NO:___)

It is understood that the above constructs are examples and one skilled in the art may readily obtain other forms of OPG binding protein using the general methodology presented her.

Growth of transfected E. coli 393, induction of OPG binding protein expression and isolation of inclusion bodies containing OPG binding protein is done according to procedures described in U.S. Serial No. 08/577,788 filed December 22, 1995. Subsequent purification of OPG binding proteins expressed in E. coli requires solubilization of bacteria inclusion bodies and renaturing of OPG binding protein using procedures available to one skilled in the art.

While the present invention has been described in terms of the preferred embodiments, it is understood that variations and modifications will occur to those skilled in the art. Therefore, it is intended that the appended claims cover all such equivalent variations which come within the scope of the invention as claimed.

WHAT IS CLAIMED IS:

1. An isolated nucleic acid encoding an osteoprotegerin binding protein selected from the group consisting of:

a) the nucleic acid sequence as in Figure 1 (SEQ ID NO:__);

b) nucleic acids which hybridize to the polypeptide coding regions as shown in Figure 1 (SEQ ID NO:__) and remain hybridized under high stringency conditions; and

c) nucleic acids which are degenerate to the nucleic acids of (a) or (b).

2. The nucleic acid of Claim 1 which is cDNA, genomic DNA, synthetic DNA or RNA.

3. A polypeptide encoded by the nucleic acid of Claim 1.

4. The nucleic acid of Claim 1 including one or more codons preferred for Escherichia coli expression.

5. The nucleic acid of Claim 1 having a detectable label attached thereto.

6. The nucleic acid of Claim 1 comprising the polypeptide-coding region of residues 1-316 as shown in Figure 1 (SEQ ID NO:__).

7. A nucleic acid encoding a polypeptide having the amino acid sequence of residues 1-316 or residues 70-316 as shown in Figure 1 (SEQ ID NO: ___).

8. An expression vector comprising the nucleic acid of Claim 1.

9. The expression vector of Claim 8 wherein the nucleic acid comprises the polypeptide-encoding region as shown in Figure 1 (SEQ ID NO:__).

10. A host cell transformed or transfected with the expression vector of Claim 8.

11. The host cell of Claim 10 which is a eucaryotic or procaryotic cell.

12. The host cell of Claim 11 which is Escherichia coli.

13. A process for the production of an osteoprotegerin binding protein comprising:

growing under suitable nutrient conditions host cells transformed or transfected with the nucleic acid of Claim 1; and

isolating the polypeptide product of the expression of the nucleic acid.

14. A polypeptide produced by the process of Claim 13.

15. A purified and isolated osteoprotegerin binding protein, or fragment, analog, or derivative thereof.

16. The protein of Claim 15 which is a human osteoprotegerin.

17. The protein of Claim 15 having the amino acid sequence as shown in Figure 1 (SEQ ID NO:__).

18. The protein of Claim 15 which has been covalently modified with a water-soluble polymer.

19. The protein of Claim 18 wherein the polymer is polyethylene glycol.

20. The protein of Claim 15 which is a soluble osteoprotegerin binding protein.

21. The protein of Claim 20 having the amino acid sequence from residues 70-316 inclusive as shown in Figure 1 (SEQ ID NO: ___), or a fragment, analog, or derivative thereof.

22. An antibody or fragment thereof which specifically binds an osteoprotegerin binding protein.

23. The antibody of Claim 22 which is a monoclonal antibody.

24. A method for detecting the presence of an osteoprotegerin binding protein in a biological sample comprising:

incubating the sample with the antibody of Claim 22 under conditions that allow binding of the antibody to the osteoprotegerin binding protein; and

detecting the bound antibody.

25. A method for detecting the presence of osteoprotegerin in a biological sample comprising:

incubating the sample with an osteoprotegerin binding protein under conditions that allow binding of the protein to osteoprotegerin; and

measuring the bound osteoprotegerin binding protein.

26. A method to assess the ability of a candidate compound to bind to an osteoprotegerin binding protein comprising:

incubating the osteoprotegerin binding protein with the candidate compound under conditions that allow binding; and

measuring the bound compound.

27. The method of Claim 26 wherein the compound is an agonist or an antagonist of an osteoprotegerin binding protein.

28. A method of regulating expression of an osteoprotegerin binding protein in an animal comprising administering to the animal a nucleic acid complementary to the nucleic acids as shown in Figure 1 (SEQ ID NO:__).

29. A pharmaceutical composition comprising a therapeutically effective amount of an osteoprotegerin binding protein in a pharmaceutically acceptable carrier, adjuvant, solubilizer, stabilizer and/or anti-oxidant.

30. The composition of Claim 29 wherein the osteoprotegerin binding protein is a human osteoprotegerin binding protein.

31. A method of treating bone disease in a mammal comprising administering a therapeutically effective amount of a modulator of an osteoprotegerin binding protein.

32. The method of Claim 31 wherein the modulator is a soluble form of an osteoprotegerin binding protein.

33. The method of Claim 32 wherein the modulator is an antibody, or fragment thereof, which specifically binds an osteoprotegerin binding protein.

ABSTRACT OF THE INVENTION

A novel polypeptide, osteoprotegerin binding protein, involved in osteolcast maturation has been identified based upon its affinity for osteoprotegerin. Nucleic acid sequences encoding the polypeptide, or a fragment, analog or derivative thereof, vectors and host cells for production, methods of preparing osteoprotegerin binding protein, and binding assays are also described. Compositions and methods for the treatment of bone diseases such as osteoporosis, bone loss due to arthritis or metastasis, hypercalcemia, and Paget's disease are also provided.

Copyright Amgen Inc. 2004

FIG.1A

```
GAGCTCGGAT CCACTACTCG ACCCACGCGT CCGGCCAGGA CCTCTGTGAA CCGGTCGGGG     60

CGGGGGCCGC CTGGCCGGGA GTCTGCTCGG CGGTGGGTGG CCGAGGAAGG GAGAGAACGA    120

TCGCGGAGCA GGGCGCCCGA ACTCCGGGCG CCGCGCC ATG CGC CGG GCC AGC CGA     175
                                         Met Arg Arg Ala Ser Arg
                                          1               5

GAC TAC GGC AAG TAC CTG CGC AGC TCG GAG GAG ATG GGC AGC GGC CCC      223
Asp Tyr Gly Lys Tyr Leu Arg Ser Ser Glu Glu Met Gly Ser Gly Pro
            10                  15                  20

GGC GTC CCA CAC GAG GGT CCG CTG CAC CCC GCG CCT TCT GCA CCG GCT      271
Gly Val Pro His Glu Gly Pro Leu His Pro Ala Pro Ser Ala Pro Ala
        25                  30                  35
```

FIG.1B

```
CCG GCG CCG CCA CCC GCC GCC TCC CGC TCC ATG TTC CTG GCC CTC CTG          319
Pro Ala Pro Pro Pro Ala Ala Ser Arg Ser Met Phe Leu Ala Leu Leu
     40                  45                  50

GGG CTG GGA CTG GGC CAG GTG GTC TGC AGC ATC GCT CTG TTC CTG TAC          367
Gly Leu Gly Leu Gly Gln Val Val Cys Ser Ile Ala Leu Phe Leu Tyr
 55                  60                  65                  70

TTT CGA GCG CAG ATG GAT CCT AAC AGA ATA TCA GAA GAC AGC ACT CAC          415
Phe Arg Ala Gln Met Asp Pro Asn Arg Ile Ser Glu Asp Ser Thr His
                 75                  80                  85

TGC TTT TAT AGA ATC CTG AGA CTC CAT GAA AAC GCA GGT TTG CAG GAC          463
Cys Phe Tyr Arg Ile Leu Arg Leu His Glu Asn Ala Gly Leu Gln Asp
             90                  95                 100

TCG ACT CTG GAG AGT GAA GAC ACA CTA CCT GAC TCC TGC AGG AGG ATG          511
Ser Thr Leu Glu Ser Glu Asp Thr Leu Pro Asp Ser Cys Arg Arg Met
        105                 110                 115
```

FIG.1C

```
AAA CAA GCC TTT CAG GGG GCC GTG CAG AAG GAA CTG CAA CAC ATT GTG          559
Lys Gln Ala Phe Gln Gly Ala Val Gln Lys Glu Leu Gln His Ile Val
    120                 125                 130

GGG CCA CAG CGC TTC TCA GGA GCT CCA GCT ATG ATG GAA GGC TCA TGG          607
Gly Pro Gln Arg Phe Ser Gly Ala Pro Ala Met Met Glu Gly Ser Trp
135                 140                 145                 150

TTG GAT GTG GCC CAG CGA GGC AAG CCT GAG GCC CAG CCA TTT GCA CAC          655
Leu Asp Val Ala Gln Arg Gly Lys Pro Glu Ala Gln Pro Phe Ala His
                155                 160                 165

CTC ACC ATC AAT GCT GCC AGC ATC CCA TCG GGT TCC CAT AAA GTC ACT          703
Leu Thr Ile Asn Ala Ala Ser Ile Pro Ser Gly Ser His Lys Val Thr
            170                 175                 180
```

FIG.1D

```
CTG TCC TCT TGG TAC CAC GAT CGA GGC TGG GCC AAG ATC TCT AAC ATG        751
Leu Ser Ser Trp Tyr His Asp Arg Gly Trp Ala Lys Ile Ser Asn Met
        185                 190                 195

ACG TTA AGC AAC GGA AAA CTA AGG GTT AAC CAA GAT GGC TTC TAT TAC        799
Thr Leu Ser Asn Gly Lys Leu Arg Val Asn Gln Asp Gly Phe Tyr Tyr
    200                 205                 210

CTG TAC GCC AAC ATT TGC TTT CGG CAT CAT GAA ACA TCG GGA AGC GTA        847
Leu Tyr Ala Asn Ile Cys Phe Arg His His Glu Thr Ser Gly Ser Val
215                 220                 225                 230

CCT ACA GAC TAT CTT CAG CTG ATG GTG TAT GTC GTT AAA ACC AGC ATC        895
Pro Thr Asp Tyr Leu Gln Leu Met Val Tyr Val Val Lys Thr Ser Ile
                235                 240                 245
```

FIG.1E

```
AAA ATC CCA AGT TCT CAT AAC CTG ATG AAA GGA GGG AGC ACG AAA AAC          943
Lys Ile Pro Ser Ser His Asn Leu Met Lys Gly Gly Ser Thr Lys Asn
            250                 255                 260

TGG TCG GGC AAT TCT GAA TTC CAC TTT TAT TCC ATA AAT GTT GGG GGA          991
Trp Ser Gly Asn Ser Glu Phe His Phe Tyr Ser Ile Asn Val Gly Gly
        265                 270                 275

TTT TTC AAG CTC CGA GCT GGT GAA GAA ATT AGC ATT CAG GTG TCC AAC         1039
Phe Phe Lys Leu Arg Ala Gly Glu Glu Ile Ser Ile Gln Val Ser Asn
    280                 285                 290

CCT TCC CTG CTG GAT CCG GAT CAA GAT GCG ACG TAC TTT GGG GCT TTC         1087
Pro Ser Leu Leu Asp Pro Asp Gln Asp Ala Thr Tyr Phe Gly Ala Phe
295                 300                 305                 310

AAA GTT CAG GAC ATA GAC T GAGACTCATT TCGTGGAACA TTAGCATGGA              1136
Lys Val Gln Asp Ile Asp
            315
```

FIG.1F

```
TGTCCTAGAT GTTTGGAAAC TTCTTAAAAA ATGGATGATG TCTATACATG TGTAAGACTA    1196

CTAAGAGACA TGGCCCACGG TGTATGAAAC TCACAGCCCT CTCTCTTGAG CCTGTACAGG    1256

TTGTGTATAT GTAAAGTCCA TAGGTGATGT TAGATTCATG GTGATTACAC AACGGTTTTA    1316

CAATTTTGTA ATGATTTCCT AGAATTGAAC CAGATTGGGA GAGGTATTCC GATGCTTATG    1376

AAAAACTTAC ACGTGAGCTA TGGAAGGGGG TCACAGTCTC TGGGTCTAAC CCCTGGACAT    1436

GTGCCACTGA GAACCTTGAA ATTAAGAGGA TGCCATGTCA TTGCAAAGAA ATGATAGTGT    1496

GAAGGGTTAA GTTCTTTTGA ATTGTTACAT TGCGCTGGGA CCTGCAAATA AGTTCTTTTT    1556
```

FIG.1G

TTCTAATGAG GAGAGAAAAA TATATGTATT TTTATATAAT GTCTAAAGTT ATATTTCAGG 1616

TGTAATGTTT TCTGTGCAAA GTTTTGTAAA TTATATTTGT GCTATAGTAT TTGATTCAAA 1676

ATATTTAAAA ATGTCTCACT GTTGACATAT TTAATGTTTT AAATGTACAG ATGTATTTAA 1736

CTGGTGCACT TTGTAATTCC CCTGAAGGTA CTCGTAGCTA AGGGGGCAGA ATACTGTTTC 1796

TGGTGACCAC ATGTAGTTTA TTTCTTTATT CTTTTTAACT TAATAGAGTC TTCAGACTTG 1856

TCAAAACTAT GCAAGCAAAA TAAATAAATA AAAATAAAAT GAATACCTTG AATAATAAGT 1916

AGGATGTTGG TCACCAGGTG CCTTTCAAAT TTAGAAGCTA ATTGACTTTA GGAGCTGACA 1976

TAGCCAAAAA GGATACATAA TAGGCTACTG AAATCTGTCA GGAGTATTTA TGCAATTATT 2036

FIG. 1H

```
GAACAGGTGT CTTTTTTTAC AAGAGCTACA AATTGTAAAT TTTGTTTCTT TTTTTTCCCA    2096

TAGAAAATGT ACTATAGTTT ATCAGCCAAA AAACAATCCA CTTTTTAATT TAGTGAAAGT    2156

TATTTTATTA TACTGTACAA TAAAAGCATT GTCTCTGAAT GTTAATTTTT TGGTACAAAA    2216

AATAAATTTG TACGAAAACC TGAAAAAAAA AAAAAAAAAA AAAAAAAGGG CGGCCGCTCT    2276

AGAGGGCCCT ATTCTATAG                                                 2295
```

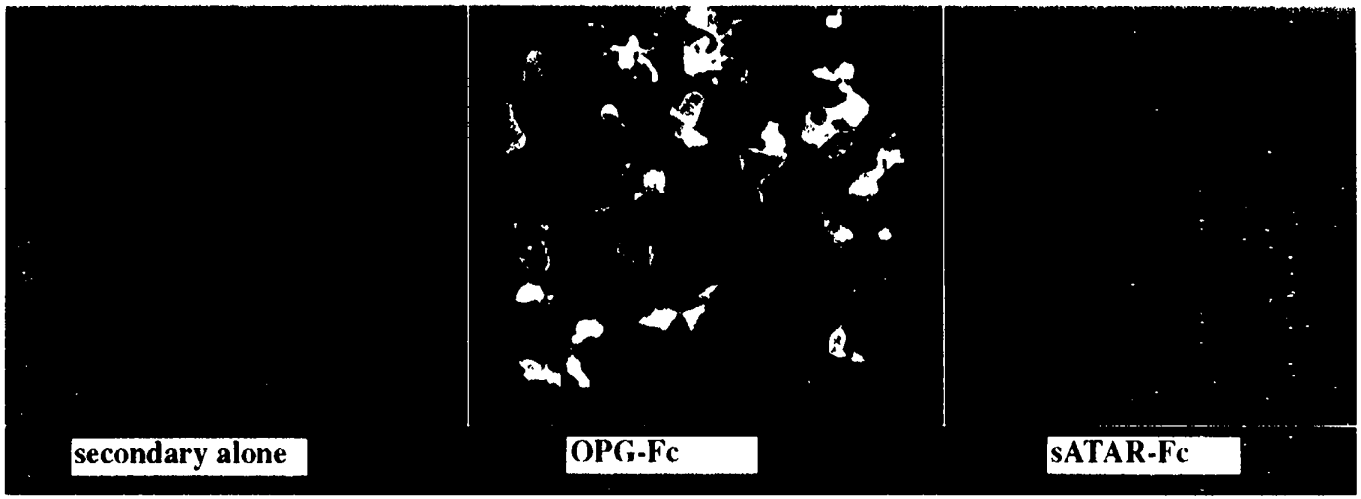


FIG. 2

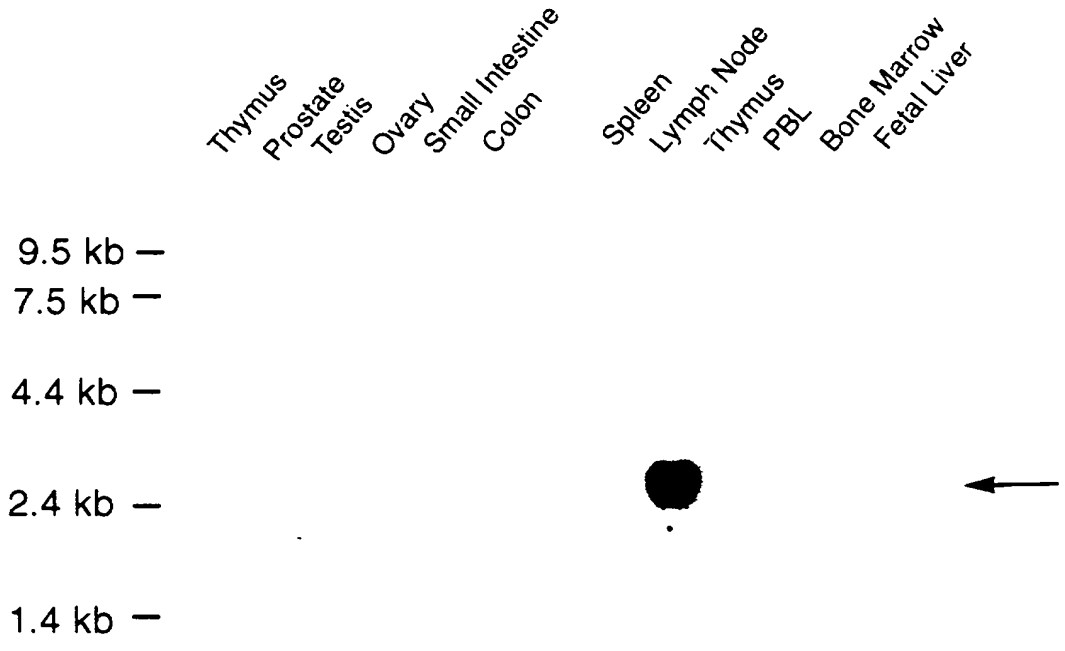

Thymus
Prostate
Testis
Ovary
Small Intestine
Colon
Spleen
Lymph Node
Thymus
PBL
Bone Marrow
Fetal Liver
9.5 kb
7.5 kb
4.4 kb
2.4 kb
1.4 kb

FIG. 3

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Boyle, William J.

(ii) TITLE OF INVENTION: OSTEOPROTEGERIN BINDING PROTEINS

(iii) NUMBER OF SEQUENCES: 7

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: Amgen Inc.
(B) STREET: 1840 Dehavilland Drive
(C) CITY: Thousand Oaks
(D) STATE: California
(E) COUNTRY: USA
(F) ZIP: 91230-1789

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Winter, Robert B.
(C) REFERENCE/DOCKET NUMBER: A-451D

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 52 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

GTTCTCCTCA TATGGATCCA AACCGTATTT CTGAAGACAG CACTCACTGC TT 52

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 37 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

TACGCACTCC GCGGTTAGTC TATGTCCTGA ACTTTGA 37

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 53 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

GTTCTCCTCA TATGAAACCT GAAGCTCAAC CATTTGCACA CCTCACCATC AAT 53

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 45 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

GTTCTCCTCA TATGCATTTA ACTATTAACG CTGCATCTAT CCCAT 45

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 59 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

GTTCTCCTCA TATGACTATT AACGCTGCAT CTATCCCATC GGGTTCCCAT AAAGTCACT 59

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2295 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 158..1105

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

```
GAGCTCGGAT CCACTACTCG ACCCACGCGT CCGGCCAGGA CCTCTGTGAA CCGGTCGGGG      60

CGGGGGCCGC CTGGCCGGGA GTCTGCTCGG CGGTGGGTGG CCGAGGAAGG GAGAGAACGA     120

TCGCGGAGCA GGGCGCCCGA ACTCCGGGCG CCGCGCC ATG CGC CGG GCC AGC CGA      175
                                          Met Arg Arg Ala Ser Arg
                                            1               5

GAC TAC GGC AAG TAC CTG CGC AGC TCG GAG GAG ATG GGC AGC GGC CCC      223
Asp Tyr Gly Lys Tyr Leu Arg Ser Ser Glu Glu Met Gly Ser Gly Pro
            10                  15                  20

GGC GTC CCA CAC GAG GGT CCG CTG CAC CCC GCG CCT TCT GCA CCG GCT      271
Gly Val Pro His Glu Gly Pro Leu His Pro Ala Pro Ser Ala Pro Ala
        25                  30                  35

CCG GCG CCG CCA CCC GCC GCC TCC CGC TCC ATG TTC CTG GCC CTC CTG      319
Pro Ala Pro Pro Pro Ala Ala Ser Arg Ser Met Phe Leu Ala Leu Leu
    40                  45                  50

GGG CTG GGA CTG GGC CAG GTG GTC TGC AGC ATC GCT CTG TTC CTG TAC      367
Gly Leu Gly Leu Gly Gln Val Val Cys Ser Ile Ala Leu Phe Leu Tyr
55                  60                  65                  70

TTT CGA GCG CAG ATG GAT CCT AAC AGA ATA TCA GAA GAC AGC ACT CAC      415
Phe Arg Ala Gln Met Asp Pro Asn Arg Ile Ser Glu Asp Ser Thr His
                75                  80                  85

TGC TTT TAT AGA ATC CTG AGA CTC CAT GAA AAC GCA GGT TTG CAG GAC      463
Cys Phe Tyr Arg Ile Leu Arg Leu His Glu Asn Ala Gly Leu Gln Asp
            90                  95                  100

TCG ACT CTG GAG AGT GAA GAC ACA CTA CCT GAC TCC TGC AGG AGG ATG      511
Ser Thr Leu Glu Ser Glu Asp Thr Leu Pro Asp Ser Cys Arg Arg Met
        105                 110                 115

AAA CAA GCC TTT CAG GGG GCC GTG CAG AAG GAA CTG CAA CAC ATT GTG      559
Lys Gln Ala Phe Gln Gly Ala Val Gln Lys Glu Leu Gln His Ile Val
    120                 125                 130

GGG CCA CAG CGC TTC TCA GGA GCT CCA GCT ATG ATG GAA GGC TCA TGG      607
Gly Pro Gln Arg Phe Ser Gly Ala Pro Ala Met Met Glu Gly Ser Trp
135                 140                 145                 150

TTG GAT GTG GCC CAG CGA GGC AAG CCT GAG GCC CAG CCA TTT GCA CAC      655
Leu Asp Val Ala Gln Arg Gly Lys Pro Glu Ala Gln Pro Phe Ala His
                155                 160                 165

CTC ACC ATC AAT GCT GCC AGC ATC CCA TCG GGT TCC CAT AAA GTC ACT      703
Leu Thr Ile Asn Ala Ala Ser Ile Pro Ser Gly Ser His Lys Val Thr
            170                 175                 180

CTG TCC TCT TGG TAC CAC GAT CGA GGC TGG GCC AAG ATC TCT AAC ATG      751
Leu Ser Ser Trp Tyr His Asp Arg Gly Trp Ala Lys Ile Ser Asn Met
        185                 190                 195

ACG TTA AGC AAC GGA AAA CTA AGG GTT AAC CAA GAT GGC TTC TAT TAC      799
Thr Leu Ser Asn Gly Lys Leu Arg Val Asn Gln Asp Gly Phe Tyr Tyr
    200                 205                 210
```

CTG TAC GCC AAC ATT TGC TTT CGG CAT CAT GAA ACA TCG GGA AGC GTA 847
Leu Tyr Ala Asn Ile Cys Phe Arg His His Glu Thr Ser Gly Ser Val
215 220 225 230

CCT ACA GAC TAT CTT CAG CTG ATG GTG TAT GTC GTT AAA ACC AGC ATC 895
Pro Thr Asp Tyr Leu Gln Leu Met Val Tyr Val Val Lys Thr Ser Ile
235 240 245

AAA ATC CCA AGT TCT CAT AAC CTG ATG AAA GGA GGG AGC ACG AAA AAC 943
Lys Ile Pro Ser Ser His Asn Leu Met Lys Gly Gly Ser Thr Lys Asn
250 255 260

TGG TCG GGC AAT TCT GAA TTC CAC TTT TAT TCC ATA AAT GTT GGG GGA 991
Trp Ser Gly Asn Ser Glu Phe His Phe Tyr Ser Ile Asn Val Gly Gly
265 270 275

TTT TTC AAG CTC CGA GCT GGT GAA GAA ATT AGC ATT CAG GTG TCC AAC 1039
Phe Phe Lys Leu Arg Ala Gly Glu Glu Ile Ser Ile Gln Val Ser Asn
280 285 290

CCT TCC CTG CTG GAT CCG GAT CAA GAT GCG ACG TAC TTT GGG GCT TTC 1087
Pro Ser Leu Leu Asp Pro Asp Gln Asp Ala Thr Tyr Phe Gly Ala Phe
295 300 305 310

AAA GTT CAG GAC ATA GAC TGAGACTCAT TTCGTGGAAC ATTAGCATGG 1135
Lys Val Gln Asp Ile Asp
315

ATGTCCTAGA TGTTTGGAAA CTTCTTAAAA AATGGATGAT GTCTATACAT GTGTAAGACT 1195

ACTAAGAGAC ATGGCCCACG GTGTATGAAA CTCACAGCCC TCTCTCTTGA GCCTGTACAG 1255

GTTGTGTATA TGTAAAGTCC ATAGGTGATG TTAGATTCAT GGTGATTACA CAACGGTTTT 1315

ACAATTTTGT AATGATTTCC TAGAATTGAA CCAGATTGGG AGAGGTATTC CGATGCTTAT 1375

GAAAAACTTA CACGTGAGCT ATGGAAGGGG GTCACAGTCT CTGGGTCTAA CCCCTGGACA 1435

TGTGCCACTG AGAACCTTGA AATTAAGAGG ATGCCATGTC ATTGCAAAGA AATGATAGTG 1495

TGAAGGGTTA AGTTCTTTTG AATTGTTACA TTGCGCTGGG ACCTGCAAAT AAGTTCTTTT 1555

TTTCTAATGA GGAGAGAAAA ATATATGTAT TTTTATATAA TGTCTAAAGT TATATTTCAG 1615

GTGTAATGTT TTCTGTGCAA AGTTTTGTAA ATTATATTTG TGCTATAGTA TTTGATTCAA 1675

AATATTTAAA AATGTCTCAC TGTTGACATA TTTAATGTTT TAAATGTACA GATGTATTTA 1735

ACTGGTGCAC TTTGTAATTC CCCTGAAGGT ACTCGTAGCT AAGGGGGCAG AATACTGTTT 1795

CTGGTGACCA CATGTAGTTT ATTTCTTTAT TCTTTTTAAC TTAATAGAGT CTTCAGACTT 1855

GTCAAAACTA TGCAAGCAAA ATAAATAAAT AAAAATAAAA TGAATACCTT GAATAATAAG 1915

TAGGATGTTG GTCACCAGGT GCCTTTCAAA TTTAGAAGCT AATTGACTTT AGGAGCTGAC 1975

ATAGCCAAAA AGGATACATA ATAGGCTACT GAAATCTGTC AGGAGTATTT ATGCAATTAT 2035

TGAACAGGTG TCTTTTTTTA CAAGAGCTAC AAATTGTAAA TTTTGTTTCT TTTTTTTCCC 2095

ATAGAAAATG TACTATAGTT TATCAGCCAA AAAACAATCC ACTTTTTAAT TTAGTGAAAG 2155

TTATTTTATT ATACTGTACA ATAAAAGCAT TGTCTCTGAA TGTTAATTTT TTGGTACAAA 2215

AAATAAATTT GTACGAAAAC CTGAAAAAAA AAAAAAAAAA AAAAAAAAGG GCGGCCGCTC 2275

TAGAGGGCCC TATTCTATAG 2295

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 316 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

```
Met Arg Arg Ala Ser Arg Asp Tyr Gly Lys Tyr Leu Arg Ser Ser Glu
 1               5                  10                  15

Glu Met Gly Ser Gly Pro Gly Val Pro His Glu Gly Pro Leu His Pro
            20                  25                  30

Ala Pro Ser Ala Pro Ala Pro Ala Pro Pro Pro Ala Ala Ser Arg Ser
        35                  40                  45

Met Phe Leu Ala Leu Leu Gly Leu Gly Leu Gly Gln Val Val Cys Ser
    50                  55                  60

Ile Ala Leu Phe Leu Tyr Phe Arg Ala Gln Met Asp Pro Asn Arg Ile
65                  70                  75                  80

Ser Glu Asp Ser Thr His Cys Phe Tyr Arg Ile Leu Arg Leu His Glu
                85                  90                  95

Asn Ala Gly Leu Gln Asp Ser Thr Leu Glu Ser Glu Asp Thr Leu Pro
            100                 105                 110

Asp Ser Cys Arg Arg Met Lys Gln Ala Phe Gln Gly Ala Val Gln Lys
        115                 120                 125

Glu Leu Gln His Ile Val Gly Pro Gln Arg Phe Ser Gly Ala Pro Ala
    130                 135                 140

Met Met Glu Gly Ser Trp Leu Asp Val Ala Gln Arg Gly Lys Pro Glu
145                 150                 155                 160

Ala Gln Pro Phe Ala His Leu Thr Ile Asn Ala Ala Ser Ile Pro Ser
                165                 170                 175

Gly Ser His Lys Val Thr Leu Ser Ser Trp Tyr His Asp Arg Gly Trp
            180                 185                 190

Ala Lys Ile Ser Asn Met Thr Leu Ser Asn Gly Lys Leu Arg Val Asn
        195                 200                 205

Gln Asp Gly Phe Tyr Tyr Leu Tyr Ala Asn Ile Cys Phe Arg His His
    210                 215                 220

Glu Thr Ser Gly Ser Val Pro Thr Asp Tyr Leu Gln Leu Met Val Tyr
225                 230                 235                 240

Val Val Lys Thr Ser Ile Lys Ile Pro Ser Ser His Asn Leu Met Lys
                245                 250                 255

Gly Gly Ser Thr Lys Asn Trp Ser Gly Asn Ser Glu Phe His Phe Tyr
            260                 265                 270

Ser Ile Asn Val Gly Gly Phe Phe Lys Leu Arg Ala Gly Glu Glu Ile
        275                 280                 285
```

Ser Ile Gln Val Ser Asn Pro Ser Leu Leu Asp Pro Asp Gln Asp Ala
290 295 300

Thr Tyr Phe Gly Ala Phe Lys Val Gln Asp Ile Asp
305 310 315

Copy provided by USPTO

DECLARATION AND POWER OF ATTORNEY

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name.

I believe I am the original, first, and sole inventor (if only one name is listed below) or a joint inventor (if plural names are listed below) of the invention entitled

OSTEOPROTEGERIN BINDING PROTEINS

which is described and claimed in the specification which:

☒ is attached hereto.

☐ was filed on _____
as Application Serial No. _____
and was amended on _____ (if applicable)

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to the examination of this application in accordance with Title 37, Code of Federal Regulations, § 1.56(a).

Power of Attorney: As a named inventor, I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application and transact all business in the Patent and Trademark Office connected therewith:

Ron K. Levy, Registration No.: 31,539, Steven M. Odre, Registration No.: 29,094, and Robert B. Winter, Registration No.: 34,458, said attorney(s)/agent(s) to have in addition full power of revocation, including the power to revoke any power herein granted.

Please send all future correspondence to:

U.S. Patent Operations/RBW
M/S 10-1-B
AMGEN INC.
Amgen Center
1840 De Havilland Drive
Thousand Oaks, California 91320-1789

Direct Telephone Calls To:

Robert B. Winter
Attorney/Agent for Applicant(s)
Registration No.: 34,458
Phone: (805) 447- 2425
Date: April 16, 1997

EXPRESS MAIL CERTIFICATE

"Express Mail" mail labeling number: TB813595189 Date of Deposit: April 16, 1997

I hereby certify that this paper or fee is being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 C.F.R. 1.10 on the date indicated above and is addressed to Box Patent Application, Assistant Commissioner for Patents, Washington, D.C. 20231

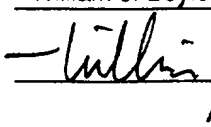
ROBERT B. WINTER
Printed Name

Signature

DECLARATION AND POWER OF ATTORNEY (cont'd)

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Full Name of Sole or First Inventor: William J. Boyle

Inventor's Signature:  Date: April 16, 1997

Residence and Post Office Address: 11678 Chestnut Ridge Street,Moorpark, CA 93021 USA
(Address, City, State, Zip Code, Country)

Citizenship: United States of America

Docket No. A-451